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From: Lawler, Michael (DPH)
Sent: Thursday, February 03, 2011 4:37 PM
To: Piro, Peter (DPH); Salemi, Charles (DPH); O'Brien, Elisabeth (DPH)
Cc: Nassif, Julianne (DPH)
Subject: RE: GHB update

Peter,
You have covered a lot of bases in your review. This all ought to be tied together with other concerns and restated to create a complete protocol for handling GHB and its analogues.

From my considerations I offer these concerns:

Determining the status of the sample upon delivery to the lab is the most critical concern of the lab. The literature reveals that the only *significant* source of GHB in the liquids we are likely to encounter is the ***fermentation product*** of red grapes; i.e., red wine, at an upper level of about 30mg/liter. Red grapes and grape juice have no detectable level of GHB. Other concerns are legitimate but moot, considering that most of our submissions probably contain sugar (fermentation substrate) which sit in the vault unrefrigerated for months before they are tested. We are not controlling for fermentation in our samples. Forget the level of GHB we see, we cannot defend that the GHB present wasn't formed in our vault. This is why we tested out the shelf life of the HPLC mobile phase, so we could keep it an ongoing stock on the shelf and test samples immediately as they came in and not store them under conditions ideal for fermentation. The samples need to be tested immediately upon arrival and unused portions refrigerated.

My literature review reveals that most of the club scene mixes we are likely to see are within the pH range of 3.7 to 3.0. The literature also reveals that this is a very stable range for measuring GHB and GBL levels. That is, within the pH range we are most likely to encounter, the conversion factors do not drive GHB or GBL equilibrium levels to extremes of mostly one or the other. By determining the pH of the sample immediately upon arrival, I believe we will see most samples at this accountable level of pH. As long as we get the pH when the sample first arrives, if it's within the anticipated range of pH 3.0 to 3.7, we can say that it was stabile and any drug detected was introduced into the liquid rather than being an artifact of its age or fermentation. Indeed, the non-destructive, established mode of HPLC at a pH of 3.0 is highly desirable. I believe we can legitimately adjust the pH of the system to suit a particular sample and control against any transesterification in our custody.

My immediate concern is that we run a response analysis to defend the most sensitive issue, that we only call positive samples that have a GHB concentration well above the highest detectable level of naturally occurring GHB, about 30 mg/L.

My ongoing recommendation is that upon arrival, samples be tested for pH, samples be prepped, tested and remaining sample be refrigerated pending its use for additional testing, as screening suggests the next step.

Finally, I would reemphasize that HPLC determines the presence of GHB with minimal manipulation compared to other approaches and is established in the authoritative text of Clarke's Analysis of Drugs and Poisons.

I believe that the *majority* of our fresh samples will arrive within a pH range of 3.7 to 3.0 and that in these samples GHB will irrefutably be of exogenous origin. Additional considerations may be necessary for samples outside this range. However, the most critical issue is immediate processing and refrigeration of the samples upon arrival.

From: Piro, Peter (DPH)
Sent: Friday, January 28, 2011 10:14 AM
To: Salemi, Charles (DPH); Lawler, Michael (DPH)
Cc: Nassif, Julianne (DPH)
Subject: GHB update

I derivatized GHB in varying concentrations (1-23 ppm) to simulate naturally occurring levels in wine. Using the normal 35:1 split, only the highest level integrated, meeting the 100,000 area count requirement. Lower concentrations did not integrate but were chromatographically visible, exceeding acquisition threshold requirement. Using an 80:1 split, the area count of the 1 mg/mL standard was reduced to slightly under 3 million. None of the dilutions integrated but all were still visible. BSTFA did successfully derivatize sugary liquids but the GHB response was noticeably reduced, hence, my concern for excessively altering the split ratio. We may have to consider using an internal standard to factor in run to run variations from tuning, injector suitability and variable injection volumes.